02-25-02 JC07 Rec'd PCT/PTO 21 FEB 2002 PCT

FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: PA-9943

U S APPLICATION NO. (IF KNOWN, SEE 37 CFR): To be assigned 10/069692

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB00/03301	August 30, 2000	August 31, 1999

TITLE OF INVENTION: **Nucleoside Analogues**

APPLICANT(S) FOR DO/EO/US: **Clifford Smith, William Cummins, Robert Nairne**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter 2 and 35 U.S.C. 1.821 - 1 825
20. ☐ A second copy of the published international application under 35 U.S C. 154(d)(4)
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

copy of this transmittal letter for charging purposes
return postcard

PCTUS1/REV03

U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/069692 To be Assigned	PCT/GB00/03301	PA-9943

24. The following fees are submitted..	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :	
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$890.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	15 - 20 =	0	x $18.00	**$0.00**
Independent claims	1 - 3 =	0	x $84.00	**$0.00**
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**

TOTAL OF ABOVE CALCULATIONS =	**$890.00**
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.	**$0.00**
SUBTOTAL =	**$890.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +	**$0.00**
TOTAL NATIONAL FEE =	**$890.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐	**$0.00**
TOTAL FEES ENCLOSED =	**$890.00**
Amount to be: refunded	$
charged	$

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **500-588** in the amount of **$890.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **500-588** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Royal N. Ronning, Jr.
Amersham Biosciences Corp.
800 Centennial Avenue
Piscataway, New Jersey 08855

(732) 457-8423

SIGNATURE

Royal N. Ronning, Jr.
NAME

32,529
REGISTRATION NUMBER

February 21, 2002
DATE

PA-9943

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	C. Smith, et al.	Group Art Unit:	To be assigned
Serial Number:	To be assigned	Examiner:	To be assigned
Filing Date:	To be assigned		
Title:	Nucleoside Analogues		

First Preliminary Amendment

Honorable Assistant Commissioner of Patents
Box Patent Application
Washington, D.C. 20231

Sir:

Please consider the following amendments and remarks in connection with the prosecution of the captioned application, which is a filing under 35 U.S.C. § 371 and claims priority to international application number PCT/GB00/03301 filed August 30, 2000. This application also claims priority to patent application number 99306887.3 filed in the EPO on August 31, 1999.

In the Claims

Please amend page 58, line 1, as follows:

[CLAIMS]

What is claimed is:

Please amend claim 3 as follows:

3. (once amended) The compound of claim 1[or claim 2], wherein a reporter moiety Rp is not present in Q.

Please amend claim 4 as follows:

4. (once amended) The compound of [any one of claims 1 to 3]claim 1, wherein the linker group Ln is a chain of 1 to 60 carbon, nitrogen, oxygen, phosphorus and/or sulphur atoms, rigid or flexible, saturated or unsaturated.

Please amend claim 5 as follows:

5. (once amended) The compound of [any one of claims 1 to 4]claim 1, wherein the reporter moiety Rp is a signal moiety or a solid surface or a reactive group by means of which a signal moiety or a solid surface may be linked to the nucleoside or nucleotide analogue.

Please amend claim 7 as follows:

7. (once amended) A nucleoside analogue comprising a compound according to [any one of claims 1 to 6]claim 1.

Please amend claim 8 as follows:

8. (once amended) A nucleotide analogue comprising a compound according to [any one of claims 2 to 6]claim 2.

Please amend claim 11 as follows:

11. (once amended) [A]The polynucleotide chain according to claim 10 wherein Q is a nucleic acid backbone consisting of sugar-phosphate repeats or modified sugar-phosphate repeats (LNA), or a backbone analogue such as peptide or polyamide nucleic acid (PNA).

Please amend claim 12 as follows:

12. (once amended) A chain extension method which comprises reacting [a]the polynucleotide chain according to [claims 10 or 11]claim 10 with a primer in the presence of a polymerase.

Please amend claim 14 as follows:

14. (once amended) A method of detecting a nucleic acid which contains a compound according to [any of claims 1 to 6]claim 1, which method comprises the step of detecting the presence of the reporter moiety Rp.

Please amend claim 15 as follows:

15. (once amended) [A]The method as claimed in claim 14 in which the reporter moiety is a radioisotope, a stable isotope, a signal moiety or a specific chemical moiety suitable for detecting by spectroscopy, especially mass spectroscopy.

In the Abstract

Please add the following abstract on a separate sheet:

-- Abstract

Compounds having structure (I) where X is CH or N, Y is -CO-, -CONW-, -O-, -S-, -SO_2-, -NWCO-, -NW-, or -OCO-, W is the same or different at different places in the molecule and each is H or alkyl or aryl or Rp or -Ln-Rp, Ln is a linker group, Rp is a reporter moiety, and Q is a sugar or a sugar analogue or a nucleic acid backbone analogue, provided that at least one reporter moiety Rp is present, provide nucleoside triphosphates which are good enzyme substrates. --

Remarks

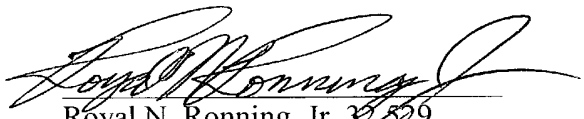
Claims 1-15 are pending in the instant application. Applicants have amended claims 2, 3, 4, 7, 8, 11, 12, 14, and 15 to more fully conform with U.S. practice and to delete multiple dependencies. A version of the claims marked up to show the amendments, as well as a clean version of the claims encompassing the amendments, is attached hereto.

Applicants also request that the attached abstract be added to the specification on a separate sheet as required.

Applicants respectfully assert that all amendments are fairly based on the specification, and respectfully request their entry.

Applicants believe that the claims, as amended, are in allowable form, and earnestly solicit the allowance of claims 1-15.

Respectfully submitted,



Royal N. Ronning, Jr. 32,529
Attorney for Applicants

Amersham Biosciences Corp.
800 Centennial Avenue
P. O. Box 1327
Piscataway, New Jersey 08855-1327

Tel: (732) 457-8423
Fax: (732) 457-8463

H:\DEPT\Law_Dept\IP Dept\Amendments\First Preliminary Amendment-PA9943 (2-21-02) doc

Claims (marked-up version showing amendment(s))

Page 58, line 1:

[CLAIMS]

What is claimed is:

3. (once amended) The compound of claim 1[or claim 2], wherein a reporter moiety Rp is not present in Q.

4. (once amended) The compound of [any one of claims 1 to 3]claim 1, wherein the linker group Ln is a chain of 1 to 60 carbon, nitrogen, oxygen, phosphorus and/or sulphur atoms, rigid or flexible, saturated or unsaturated.

5. (once amended) The compound of [any one of claims 1 to 4]claim 1, wherein the reporter moiety Rp is a signal moiety or a solid surface or a reactive group by means of which a signal moiety or a solid surface may be linked to the nucleoside or nucleotide analogue.

7. (once amended) A nucleoside analogue comprising a compound according to [any one of claims 1 to 6]claim 1.

8. (once amended) A nucleotide analogue comprising a compound according to [any one of claims 2 to 6]claim 2.

11. (once amended) [A]The polynucleotide chain according to claim 10 wherein Q is a nucleic acid backbone consisting of sugar-phosphate repeats or modified sugar-phosphate repeats (LNA), or a backbone analogue such as peptide or polyamide nucleic acid (PNA).

12. (once amended) A chain extension method which comprises reacting [a]the polynucleotide chain according to [claims 10 or 11]claim 10 with a primer in the presence of a polymerase.

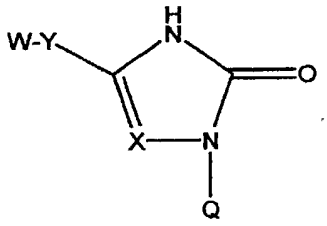
14. (once amended) A method of detecting a nucleic acid which contains a compound according to [any of claims 1 to 6]claim 1, which method comprises the step of detecting the presence of the reporter moiety Rp.

15. (once amended) [A]The method as claimed in claim 14 in which the reporter moiety is a radioisotope, a stable isotope, a signal moiety or a specific chemical moiety suitable for detecting by spectroscopy, especially mass spectroscopy.

Claims (clean version encompassing amendments)

What is claimed is:

1. A compound having the structure



where X is CH or N,

Y is –CO–, –CONW–, –O–, –S–, –SO–, –SO_2–, –NWCO–, –NW–, or –OCO–,

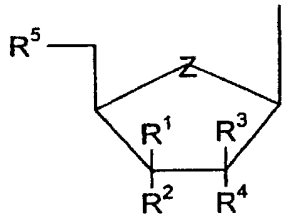
W is the same or different at different places in the molecule and each is H or alkyl or aryl or Rp or –Ln–Rp,

Ln is a linker group,

Rp is a reporter moiety, and

Q is a sugar or a sugar analogue or a nucleic acid backbone or backbone analogue, provided that at least one reporter moiety Rp is present.

2. The compound as claimed in claim 1, wherein Q is



where Z is O, S, Se, SO, NW or CH_2,

R^1, R^2, R^3 and R^4 are the same or different and each is H,

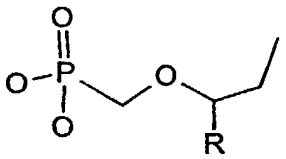
OH, F, NH_2, N_3, O-hydrocarbyl or Rp or –Ln–Rp,

R^5 is OH, SH or NH_2 or mono–, di– or tri-phosphate or –thiophosphate, or corresponding boranophosphate,

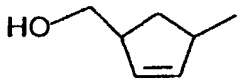
or one of R^2 and R^5 is a phosphoramidite or other group for incorporation in a polynucleotide chain, or a reporter moiety,

or Q consists of one of the following modified sugar structures

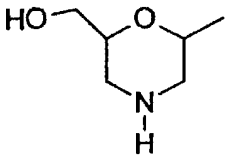
Acyclic Sugars

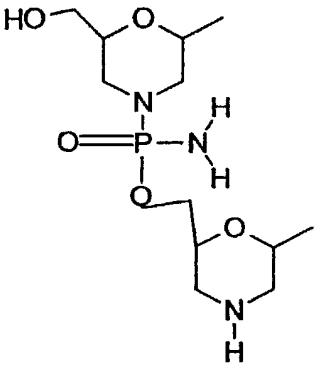


R= CH_3, CH_2OH, H,



Morpholino Backbone





3. (once amended) The compound of claim 1, wherein a reporter moiety Rp is not present in Q.

4. (once amended) The compound of claim 1, wherein the linker group Ln is a chain of 1 to 60 carbon, nitrogen, oxygen, phosphorus and/or sulphur atoms, rigid or flexible, saturated or unsaturated.

5. (once amended) The compound of claim 1, wherein the reporter moiety Rp is a signal moiety or a solid surface or a reactive group by means of which a signal moiety or a solid surface may be linked to the nucleoside or nucleotide analogue.

6. The compound of claim 5, wherein the reactive group is NH_2, OH, COOH, $CONH_2$, ONH_2, SH, or a thiophosphate or a hydrazine or a hydrazide, or an active ester or aldehyde or maleimide.

7. (once amended) A nucleoside analogue comprising a compound according to claim 1.

8. (once amended) A nucleotide analogue comprising a compound according to claim 2.

9. The nucleotide analogue of claim 8, wherein R^5 is triphosphate.

10. A polynucleotide chain comprising a nucleoside analogue of claim 7.

11. (once amended) The polynucleotide chain according to claim 10 wherein Q is a nucleic acid backbone consisting of sugar-phosphate repeats or modified sugar-phosphate repeats (LNA), or a backbone analogue such as peptide or polyamide nucleic acid (PNA).

12. (once amended) A chain extension method which comprises reacting the polynucleotide chain according to claim 10 with a primer in the presence of a polymerase.

13. A chain extension method according to claim 12 in which the primer is chosen to hybrisise with a section of the polynucleotide chain not including the nucleoside analogue.

14. (once amended) A method of detecting a nucleic acid which contains a compound according to claim 1, which method comprises the step of detecting the presence of the reporter moiety Rp.

15. (once amended) The method as claimed in claim 14 in which the reporter moiety is a radioisotope, a stable isotope, a signal moiety or a specific chemical moiety suitable for detecting by spectroscopy, especially mass spectroscopy.

Abstract

Compounds having structure (I) where X is CH or N, Y is -CO-, -CONW-, -O-, -S-, -SO_2-, -NWCO-, -NW-, or -OCO-, W is the same or different at different places in the molecule and each is H or alkyl or aryl or Rp or -Ln-Rp, Ln is a linker group, Rp is a reporter moiety, and Q is a sugar or a sugar analogue or a nucleic acid backbone analogue, provided that at least one reporter moiety Rp is present, provide nucleoside triphosphates which are good enzyme substrates.

NUCLEOSIDE ANALOGUES

Introduction

The present invention relates to compounds suitable for use as nucleoside analogues, and to polynucleotide chains comprising nucleoside analogues.

Nucleic acids are manipulated *in vitro* in a wide variety of research and diagnostic techniques. The methods can involve the synthesis of nucleic acid probes by means of DNA or RNA polymerase, reverse transcriptase or terminal transferase enzymes for the purposes of labelling or determination of base sequence identity. Labelling often involves the incorporation of a nucleotide which is chemically labelled or which is of a particular chemical composition so as to make it detectable. Nucleic acid probes made in this way can be used to determine the presence of a nucleic acid target which has a complementary sequence by means of hybridisation of the probe to the target.

In WO 94/21658 T I Kalman describes novel nucleoside or nucleotide analogues having a 4-acetylimidazolin-2-one base and their use for inhibiting virally encoded reverse transcriptases.

In Z Naturforsch B, 1986, 41b (12), 1571-9, T Fukuda *et al* describe the effect of incorporation of nucleoside analogues having an imidazolin-2-one base as both T and G in DNA duplexes.

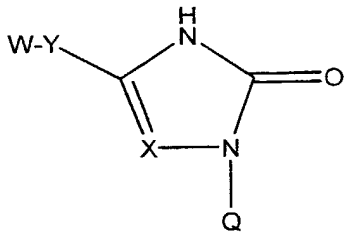
In Tetrahedron Letters, 40 (1999), 835-838, E Bedu *et al* describe the preparation of a nucleoside analogue having a 4-amidoimidazolin-2-one base and used as a cytosine analogue in triple helix forming oligonucleotides.

Purine and pyrimidine base nucleosides and nucleotides have been derivatised with reporter groups and are well known and widely used

for labelling DNA or RNA and in other molecular biology applications. But these molecules are often poor enzyme substrates. There is a continuing need for labelled nucleoside analogues whose triphosphates are good enzyme substrates.

Statement of Invention

According to the present invention there is provided a compound having the structure



where X is CH or N,

Y is -CO-, -CONW-, -O-, -S-, -SO-, -SO_2-, -NWCO-,-NW-, or -OCO-,

W is the same or different at different places in the molecule and each is H or alkyl or aryl or Rp or –Ln–Rp,

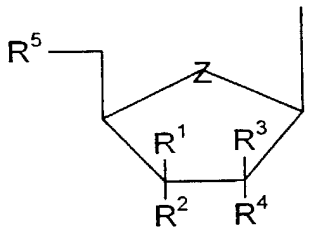
Ln is a linker group,

Rp is a reporter moiety, and

Q is a sugar or a sugar analogue or a nucleic acid backbone or backbone analogue,

provided that at least one reporter moiety Rp is present.

Q may be



where Z is O, S, Se, SO, NW or CH_2,

R^1, R^2, R^3 and R^4 are the same or different and each is H,

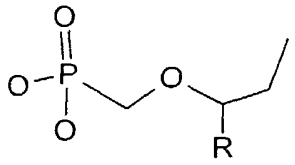
OH, F, NH_2, N_3, O-hydrocarbyl or Rp or –Ln–Rp,

R^5 is OH, SH or NH_2 or mono-, di- or tri-phosphate or -thiophosphate, or corresponding boranophosphate,

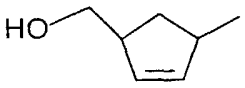
or one of R^2 and R^5 is a phosphoramidite or other group for incorporation in a polynucleotide chain, or Rp or –Ln–Rp,

or Q consists of one of the following modified sugar structures

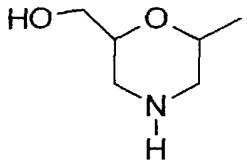
Acyclic Sugars

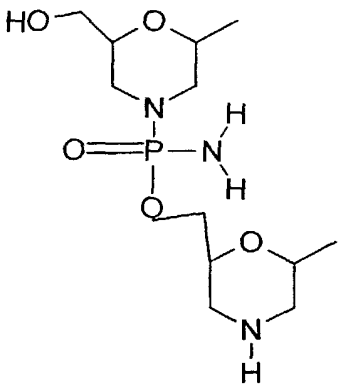


R= CH_3 , CH_2OH , H ,



Morpholino Backbone





or Q is a nucleic acid backbone consisting of sugar-phosphate repeats or modified sugar-phosphate repeats (e.g. LNA) (Koshkin *et al*, 1998, Tetrahedron 54, 3607-30) or a backbone analogue such as peptide or polyamide nucleic acid (PNA). (Nielsen *et al*, 1991, Science 254, 1497 - 1500).

The reporter moiety Rp, with or without the linker group Ln, will be present in W or Y when Y contains W and will always have at least one atom between the reporter and the base ring. When Q is a sugar or sugar analogue or a modified sugar, these compounds are nucleotide analogues or nucleoside analogues. When Q is a nucleic acid backbone or a backbone analogue, these compounds are herein called nucleic acids or polynucleotides.

A nucleoside analogue is a molecule which is capable of being incorporated, either chemically or enzymatically, into an oligomeric or polymeric nucleic acid (DNA or RNA) chain, and when so incorporated of

forming a base pair with a nucleotide residue in a complementary chain or base stacking in the appropriate nucleic acid chain.

In the context of this invention, a nucleotide is a naturally occurring compound comprising a heterocyclic base and a sugar moiety including a phosphate. A nucleoside is a corresponding compound in which a phosphate is not present. Nucleotide analogues and nucleoside analogues are analogous compounds having different bases and/or different sugar moieties. A nucleoside analogue is a compound which is capable of forming part of a nucleic acid (DNA or RNA or PNA) chain, and is there capable of base-pairing with a base in a complementary chain or base stacking in the appropriate nucleic acid chain. A nucleoside analogue may be specific, by pairing with only one complementary nucleotide; or degenerate, by base pairing with more than one of the natural bases, e.g. with pyrimidines (T/C) or purines (A/G); or universal, by pairing with each of the natural bases with little discrimination; or it may pair with another analogue or itself.

In one preferred aspect of the invention, the base analogue is linked to a sugar moiety such as ribose, deoxyribose or dideoxyribose to form a nucleoside analogue. When the group R^5 is triphosphate, the nucleoside triphosphate analogues of the invention are capable of being incorporated by enzymatic means into nucleic acid chains.

A reporter moiety Rp may be any one of various things. It may be a radioisotope by means of which the nucleoside analogue is rendered easily detectable, for example 32-P or 33-P or 35-S incorporated in a phosphate or thiophosphate or phosphoramidite or H-phosphonate group, or alternatively 3-H or 14-C or 125-I. It may be a stable isotope or a specific chemical moiety suitable for detection by mass spectrometry. (Or the compound as a whole may be suitable for detection by mass spectrometry.) It may be a signal moiety e.g. an enzyme, hapten, fluorophore, chemiluminescent group, Raman label or electrochemical label.

The reporter moiety may be a solid surface, to which the nucleoside analogue is attached and by means of which it may be distinguished from nucleoside analogues not so immobilised. The reporter moiety may be a reactive group, either a nucleophilic group, e.g. NH_2, OH, COOH, $CONH_2$, ONH_2, SH or a thiophosphate or a hydrazine or a hydrazide, or an electrophilic group e.g. an active ester or aldehyde or maleimide, by which a signal moiety and/or a solid surface may be attached, before or after incorporation of the nucleoside analogue in a nucleic acid chain. Such reporter groups are well known and well described in the literature.

A linker group Ln is a chain of 1 to 60 or more carbon, nitrogen, oxygen phosphorus and/or sulphur atoms, rigid or flexible, saturated or unsaturated, as well known in the field. Preferably the linker group is joined to a 4-triazole ring (when X is N) or to a 4-imidazole ring (when X is CH) of the nucleoside analogue molecule by a group having an alpha carbonyl group, e.g. amide or an amine bond. Preferably the linker group is joined to the reporter moiety by an amide bond.

To avoid risk of steric hindrance, a linker preferably has at least three chain atoms, e.g. $-(CH_2)_n-$ where n is at least 3.

Two (or more) reporter moieties may be present, e.g. a signal moiety and a solid surface, or a hapten and a different signal moiety, or two fluorescent signal groups to act as donor and acceptor. Various formats of these arrangements may be useful for separation or detection purposes.

Purine and pyrimidine nucleoside derivatives labelled with reporter moieties are well known and well described in the literature. Labelled nucleoside derivatives have the advantage of being readily detectable during sequencing or other molecular biology techniques.

R^1, R^2, R^3 and R^4 may each be H, OH, F, NH_2, N_3, O-alkyl or a reporter moiety. Thus ribonucleosides, and deoxyribonucleosides and dideoxyribonucleosides are envisaged together with other nucleoside analogues. These sugar substituents may contain a reporter moiety in

place of or in addition to the one or two present in the base.

R^5 is OH or mono-, di- or tri-phosphate or -thiophosphate or corresponding boranophosphate. From nucleosides (R^5 is OH) it is readily possible to make the corresponding nucleotides (R^5 is triphosphate) by literature methods. Alternatively, one of R^2 and R^5 may be a phosphoramidite or H-phosphonate or methylphosphonate or phosphorothioate or amide, or an appropriate linkage to a solid surface e.g. hemisuccinate controlled pore glass, or other group for incorporation , generally by chemical means, in a polynucleotide chain. The use of phosphoramidites and related derivatives in synthesising oligonucleotides is well known and described in the literature.

In the new nucleoside analogues to which this invention is directed, at least one reporter moiety is present preferably in the base analogue and/or optionally in the sugar moiety or a phosphate group. Reporter moieties may be introduced into the sugar moiety of a nucleoside analogue by literature methods (e.g. J. Chem. Soc. Chem. Commun. 1990, 1547-8; J. Med. Chem., 1988, 31. 2040-8). Reporter moieties in the form of isotopic labels may be introduced into phosphate groups by literature methods (Analytical Biochemistry, 214, 338-340, 1993; WO 95/15395).

When R^5 is triphosphate, the nucleoside analogues are available for enzymatic incorporation in DNA or RNA. The invention includes in another aspect the polynucleotide chain comprising at least one residue of the nucleoside analogue as defined.

Nucleoside analogues of this invention are useful for labelling DNA or RNA or for incorporating in oligonucleotides or PNA. A reporter moiety is attached at a position where it does not have a significant detrimental effect on the physical or biochemical properties of the nucleoside analogue, in particular its ability to be incorporated in single stranded or double stranded nucleic acid.

A template containing the incorporated nucleoside analogue of this invention may be suitable for copying in nucleic acid synthesis. If a

reporter moiety of the incorporated nucleoside analogue consists of a linker group, then a signal moiety can be introduced into the incorporated nucleoside analogue by being attached through a terminal or other reactive group of the linker group.

A nucleoside analogue triphosphate of this invention may be incorporated by enzymes such as terminal transferase to extend the 3' end of nucleic acid chains in a non-template directed manner. Tails of the nucleoside analogue triphosphate produced in this way may be detected directly in the absence of any reporter label by use of antibodies directed against the nucleoside analogue. The analogues when incorporated into oligonucleotides or nucleic acids may be acted upon by nucleic acid modification enzymes such as ligases or restriction endonucleases.

The nucleoside analogues of this invention can also be used in any of the existing applications which use native nucleic acid probes labelled with haptens, fluorophores or other reporter groups, for example on Southern blots, dot blots and in polyacrylamide or agarose gel based methods or solution hybridisation assays and other assays in microtitre plates or tubes or assays of oligonucleotides or nucleic acids such as on microchips. The probes may be detected with antibodies targeted either against haptens which are attached to the base analogues or against the base analogues themselves which would be advantageous in avoiding additional chemical modification. Antibodies used in this way are normally labelled with a detectable group such as a fluorophore or an enzyme. Fluorescent detection may also be used if the base analogue itself is fluorescent or if there is a fluorophore attached to the nucleoside analogue.

RNA is an extremely versatile biological molecule. Experimental studies by several laboratories have shown that in vitro selection techniques can be employed to isolate short RNA molecules from RNA libraries that bind to proteins, not normally associated with RNA binding, including a few antibodies, with high affinity and specificity (Gold, Allen, Binkley, et al,1993, 497-510 in The RNA World, Cold Spring Harbor